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STUDIES ON CHICKEN FAT.

I. THE OCCURRENCE AND PERMANENCE OF LIPASE IN THE FAT OF THE COMMON FOWL (Gallus domesticus).

By M. E. Pennington and J. S. Hepburn, *Food Research Laboratory.*

II. THE OXIDATION OF CHICKEN FAT BY MEANS OF HYDROGEN PEROXID.

By Joseph S. Hepburn, *Food Research Laboratory.*

I. THE OCCURRENCE AND PERMANENCE OF LIPASE IN THE FAT OF THE COMMON FOWL (Gallus domesticus).

Post-mortem changes in chicken fat include a pronounced increase in acidity, whether it takes place slowly, as when the fat is kept hard frozen, or more and more rapidly as the temperature rises above the congealing point of flesh.[1] Such an increase in the acidity of fats has generally been traced to the enzym lipase, which is not only widely distributed in nature, but is one of the most resistant enzyms known. Hence this study was undertaken to determine its presence in chicken fats coming from varying sources and in those which had been hard frozen for considerable lengths of time. A review of the literature on lipase [2] revealed but one record of the observation of that enzym in the common fowl; Kastle and Loevenhart [3] found lipase in the liver of the chicken.

The technique is fairly simple. The crude abdominal fat is passed several times through a meat chopper, and its acidity is determined by the method of Pennington and Hepburn.[1] A weighed sample of the ground fat is triturated in a mortar with sand and then extracted by trituration with water. The solution is poured through a strainer

[1] U. S. Dept. Agr., Bureau of Chemistry Bul. 115, 1908, p. 57; and Cir. 70, 1911; Premier Congrès Intern. du Froid, Rapports et Communications, 1908, 2: 216; J. Amer. Chem. Soc., 1910, 32: 568.

[2] Hepburn, J. Franklin Inst., 1909, 168: 429.

[3] Amer. Chem. J., 1900, 24: 491.

of wire gauze and then is filtered through absorbent cotton. The extraction is repeated several times and finally the insoluble matter is placed on a muslin filter and squeezed to remove the aqueous extract as far as possible; this filtrate also is strained through wire gauze and filtered through absorbent cotton. The quantity of water used for extraction is so regulated that the total volume of the filtrate in cubic centimeters is exactly 10 times the weight in grams of the crude fat used in making the extract.

Fifty cubic centimeters of the aqueous extract and 1 cc of an ester (ethyl acetate, butyrate, or benzoate, or amyl salicylate) are placed in an Erlenmeyer flask of 100 cc capacity; 0.2 cc of a 1 per cent solution of phenolphthalein in alcohol are added, and the solution is neutralized. One cubic centimeter of toluol is used as a bactericide, and at intervals during the incubation more is added to replace that lost by evaporation. The flask and its contents are incubated at 40° C.—the optimum temperature for lipase[2]—for periods of time varying between 24 and 168 hours, usually 72 hours. A 50 cc sample of the aqueous extract is boiled, cooled to the temperature of the room, and run as a blank experiment in exactly the same manner as the determination proper. At the end of the incubation both determination and blank experiment are titrated with tenth-normal sodium hydroxid; the increase in acidity of the determination proper over the blank is due to the action of lipase.

The data of the preliminary experiments are given in Table I, page 3. Since lipase will produce a further increase in acidity, if the solution is neutralized at intervals and then subjected to further incubation, experiments of this type were carried out as one of the tests for lipase. Thus in experiment 1021–2, in the lipolysis of ethyl butyrate, after 24 hours, the determination proper showed an acidity of 1.30 cc, and the blank of 0.15 cc; therefore the acidity due to lipase was 1.15 cc; in the second period of 24 hours, a further increase in the determination proper of 0.80 cc and in the blank of 0.20 cc occurred, giving an increase due to lipase of 0.60 cc. In the table the total hydrolysis in the total time is given.

[1] J. Amer. Chem. Soc., 1910, 32: 568. [2] Kastle and Loevenhart, loc. cit.

TABLE I.—*The occurrence of lipase in the fat of chickens.*

Description and number of sample.	Acid value of crude abdominal fat.	Ester.	Period in hours in incubator at 40° C.	Acidity expressed as cubic centimeters of tenth-normal sodium hydroxid.		
				Determination proper after incubation.	Blank after incubation.	Increase due to lipase.
No. 1023–3, well-bled, sound western market chicken of known history, at end of retail period.	2.80	Ethyl butyrate.	24	2.00	1.00	1.00
No. 1021–2, undrawn western chicken: 2 days in chillroom, 28°–36° F.; 7 days in freezer, 5°–19° F.; 7 days in refrigerator car, 31°–37° F.; 9 days at wholesaler's, 39°–43° F.; 4 days at retailer's, 28°–66° F.	3.53	Ethyl butyrate.	24	1.30	.15	1.15
			48	2.10	.35	1.75
			120	2.65	.50	2.15
			144	2.80	.50	2.30
No. 1021–3, wire-drawn western chicken: Same history as 1021–2, undrawn, with two additional days at retailer's at 28°–66° F.	2.78	Ethyl acetate...	72	1.85	.30	1.55
			96	2.10	.45	1.65
		Ethyl butyrate.	72	2.15	.35	1.80
			96	2.25	.40	1.85
		Amyl salicylate.	72	.70	.25	.45
			96	1.00	.45	.55
No. 1025–1, well-bled, sound western market chicken of known history, at beginning of retail period.	1.97	Ethyl acetate...	72	1.65	.50	1.15
			144	2.20	.60	1.60
		Ethyl butyrate.	72	.75	.20	.55
			144	.95	.30	.65
		Amyl salicylate.	72	.95	.25	.70
			144	1.50	.30	1.20
No. 343, broiling chickens of known history, hard frozen for 12½ months.	2.41	Ethyl acetate...	24	1.45	.45	1.00
			48	2.30	.80	1.50
			120	2.85	1.10	1.75
			144	3.10	1.10	2.00
			168	3.60	1.10	2.50
		Ethyl butyrate.	24	1.05	.30	.75
			48	2.05	.45	1.60
			120	2.50	.85	1.65
			144	2.65	.85	1.80
			168	3.00	1.05	1.95
		Ethyl benzoate.	24	.90	.55	.35
			48	1.90	.90	1.00
			120	3.05	1.40	1.65
			144	3.95	1.75	2.20
			168	4.95	2.05	2.90
		Amyl salicylate.	24	.50	.25	.25
			48	.90	.45	.45
			120	1.20	.55	.65
			144	2.10	.90	1.20
			168	2.40	1.10	1.30
No. 345, broiling chickens of known history, hard frozen 13 months.	2.01	Ethyl acetate...	24	1.10	.40	.70
			48	1.65	.65	1.00
		Ethyl butyrate.	24	1.00	.30	.70
			48	1.75	.45	1.30

The presence of lipase is shown by the following facts: (1) The acidity of the crude abdominal fat; (2) the hydrolysis of the various esters; (3) the further hydrolysis of the esters after neutralization during incubation; (4) the influence of chemical constitution on the rate of hydrolysis of the esters.

During both the preliminary and the final series of experiments, the rate of hydrolysis was greater in the case of the butyric ester than in that of the acetic, i. e., the rate became greater as the carbon

content of the fatty acid increased. Kastle and Loevenhart [1] have found this property to be characteristic of lipase.

The final series of experiments is a study of the comparative activity of the lipase in fresh chickens and in fowls kept for varying periods of time at different temperatures, from 5° F. as a minimum to 65°–78° F. as a maximum. Throughout the entire series the period of incubation was constant, 72 hours. The samples included a chicken which had just been killed and from which the animal heat had not been lost; one kept in the chill room at 32° F. for 24 hours; birds from the West and South of known history at various stages of their marketing, and fowls kept hard frozen for periods as long as 89 months. The chickens hard frozen for 16 months were market birds; those stored for longer periods were not marketable, and are only of scientific interest. The "green struck" chicken represents incipient putrefaction, while the bird kept at the temperature of the room for 7 days was in an advanced state of putrefaction.

In connection with this study of the action of prolonged freezing on the activity of lipase, it is interesting to review briefly the literature on the influence of low temperatures on enzym action. Macfadyen and Rowland [2] found that zymase withstands for 24 hours the temperature of liquid air, while Buchner [3] prepared the same enzym from yeast cells with the aid of solid carbon dioxid. The work of Kovchoff [4] shows that the proteolytic enzym of wheat, peas, and the tissue of *Vicia faba* retains its activity after these substances have been frozen for 24 hours. Pepsin and gastric juice are active at 0° C., as shown by the work of Müller,[5] Flaum,[6] Fick and Murisier,[7] and Hoppe-Seyler.[8] Recently Oguro [9] has demonstrated that pepsin and dilute hydrochloric acid digest ricin at temperatures of 8°, 5°, and 0° C. Müller [5] found that rennin resists a temperature of 0° C. Kastle and Loevenhart [1] state that the lipase of a pig pancreas retained 60 per cent of its power to hydrolyze ethyl butyrate after that organ had been kept in cold storage for seven days. A dry fat-free pancreas retained its lipolytic power in almost full strength for seven weeks. Even a putrefying pancreas still possessed power to split the ester to a slight extent. Richardson [10] states that the fat-splitting enzym of the pancreatic juice "does still retain and show a little activity at freezer temperatures." His experiments were made on a mixture

[1] Amer. Chem. J., 1900, **24**: 491.
[2] Lancet, 1900, **78** (1): 849; 1130.
[3] Die Zymasegärung, 1903. Résumé by Bradbury in Journal of the Franklin Institute, 1904, **157**: 41.
[4] Ber. d. bot. Ges., 1907, **25**: 473.
[5] Arch. Hyg., 1903, **47**: 127.
[6] Zts. Biol., 1891, **28** (N. F. **10**): 433.
[7] Verhandlungen der Würzburger physiologisch-medizinische Gesellschaft, 1872, N. F. **2**: 122.
[8] Pflüger's Archiv gesam. Physiol., 1877, **14**: 395.
[9] Biochem. Zts., 1909, **22**: 278.
[10] Premier Congrès International du Froid, Rapports et Communications, 1908, **2**: 315.

of fresh hog pancreas and neutral lard kept at a temperature of −9° C. to −12° C. for as long a period as three months.

TABLE II.—*Comparative activity of lipase in the fat of chickens kept under varying conditions of temperature and time.*

[Period of incubation, 72 hours at 40° C.]

Description and number of sample.	Acid value of crude abdominal fat.	Ester.	Acidity expressed as tenth-normal sodium hydroxid.		
			Determination proper after incubation.	Blank after incubation.	Increase due to lipase.
			cc.	*cc.*	*cc.*
No. 358, chicken retaining animal heat	0.20	Ethyl acetate	0.95	0.20	0.75
		Ethyl butyrate	.80	.20	.60
		Ethyl benzoate	1.25	.25	1.00
		Amyl salicylate	.40	.50	None.
No. 359, chicken in chill room at 32° F. for 24 hours.	.53	Ethyl acetate	1.35	.20	1.15
		Ethyl butyrate	2.25	.15	2.10
		Ethyl benzoate	3.10	1.85	1.25
		Amyl salicylate	.80	.20	.60
No. 2044–1, dry-packed southern market chicken of known history at end of transportation period.	1.08	Ethyl acetate	1.20	.30	.90
		Ethyl butyrate	1.90	.30	1.60
		Ethyl benzoate	3.90	2.90	1.00
		Amyl salicylate	.55	.20	.35
No. 1025–1, well-bled, sound western market chicken of known history, at beginning of retail period.	1.97	Ethyl acetate	1.65	.50	1.15
		Ethyl butyrate	.75	.20	.55
		Amyl salicylate	.95	.25	.70
No. 1021–3, wire-drawn western chicken, 2 days in chill room, 28–36° F.; 7 days in freezer, 5–19° F.; 7 days in refrigerator car, 31–37° F.; 9 days at wholesaler's, 39–43° F.; 6 days at retailer's, 28–66° F.	2.78	Ethyl acetate	1.85	.30	1.55
		Ethyl butyrate	2.15	.35	1.80
		Amyl salicylate	.70	.25	.45
No. 2053–2, dry-packed southern market chicken of known history, from commission merchant; green struck.	3.20	Ethyl acetate	2.30	.70	1.60
		Ethyl butyrate	3.15	.30	2.85
		Ethyl benzoate	3.40	3.40	.00
		Amyl salicylate	1.20	.45	.75
No. 361, chicken kept in room for 7 days; average temperature, 72.5° F.; minimum temperature, 67° F.; maximum temperature, 78° F.; advanced putrefaction.	7.14	Ethyl acetate	2.70	1.10	1.60
		Ethyl butyrate	3.50	.50	3.00
		Amyl salicylate	1.30	.50	.80
No. 1001–4, well-bled, sound, western market chicken of known history, hard frozen for 16 months.	33.13	Ethyl acetate	3.20	1.10	2.10
		Ethyl butyrate	4.50	.65	3.85
		Ethyl benzoate	5.00	1.30	3.70
		Amyl salicylate	1.95	.70	1.25
No. 1001–4, badly bled, rubbed, western market chicken of known history, hard frozen for 16 months.	34.85	Ethyl acetate	4.20	1.70	2.50
		Ethyl butyrate	5.70	1.10	4.60
No. 365, chicken of known history, hard frozen for 28 months.	36.09	Ethyl butyrate	3.05	.80	2.25
No. 367, chicken of known history, hard frozen for 29 months.	6.62	Ethyl acetate	1.40	.60	.80
		Ethyl butyrate	1.50	.40	1.10
No. 366, chicken of known history, hard frozen for 42 months.	12.78	Ethyl acetate	2.10	.85	1.25
		Ethyl butyrate	3.50	.60	2.90
No. 372, chicken hard frozen for 54 months; history previous to freezing unknown.	39.40	Ethyl acetate	5.65	2.75	2.90
		Ethyl butyrate	6.30	1.50	4.80
		Ethyl benzoate	4.20	1.80	2.40
		Amyl salicylate	8.50	4.00	4.50
No. 373, chicken hard frozen for 89 months; history previous to freezing unknown.	32.88	Ethyl acetate	4.95	2.15	2.80
		Ethyl butyrate	5.90	1.35	4.55

The results of the final series of experiments are given in Table II. The lowest acidity of the crude fat and the least activity of the lipase is in the chicken retaining the animal heat; as the birds age after death the acidity of the crude fat becomes greater, as does also the activity of the enzym; the increase is apparently dependent on both temperature and the period of keeping and has occurred even in the chicken kept in the chill room at 32° F. for 24 hours. The three experiments, 2044–1, 1025–1, and 1021–3, represent western and southern chickens in various stages of their marketing; the highest acidity of the fat and the greatest activity of the lipase is found in the sample which has been on the market for the longest time. Likewise, the acidity of the fat and the activity of the enzym are greater in the fowl in an advanced stage of putrefaction than in the "green struck" bird (incipient putrefaction). In the hard frozen samples the greatest activity of the lipase and the highest acidity of the fat is in the chicken kept hard frozen for 54 months; however, the enzym was energetic in the bird hard frozen for 89 months. Therefore, lipase is able to resist prolonged freezing, even for a period of almost seven and a half years. The rate of increase in the acidity of the crude fat and in the activity of the enzym is greatly decreased by prolonged hard freezing.

Apparently the crude fat of a chicken which has just been killed is almost, if not absolutely, neutral and contains lipase largely, if not entirely, as a zymogen, which is converted into the active form as the bird ages after death. Greater activity of the enzym and a higher acidity of the crude fat usually occur as the period of keeping grows longer. This transition from zymogen to active enzym is proved by the fact that extracts from aged birds possess greater lipolytic power than does the extract from the chicken retaining the animal heat.

Apart from the rendering active of a zymogen, an increase in the acidity of the fat may tend to increase the activity of the lipase toward the fat in situ. Thus Connstein, Hoyer, and Wartenberg,[1] who studied the lipase of the seed of the castor-oil plant (*Ricinus communis*), found that sulphuric, phosphoric, and acetic acids and sodium acid sulphate exert an accelerating action on the hydrolyses produced by lipase. However, they contend that certain of their experiments make it probable that these compounds act as catalytic agents for the active enzym without functioning as activators for a zymogen. The optimum concentration of the reagents mentioned lies between tenth normal and third normal. Acids which are insoluble in water also exert an accelerating influence, although far larger quantities of such acids must be used to obtain the same result. Moreover, these investigators also discovered that the rate

[1] Ber. d. Chem. Ges. 1902, 35: 3988.

of hydrolysis becomes greater after the lipase of *Ricinus communis* has acted on a fat for some time. Hence, contrary to the habit of most enzyms, a piling up of the enzymic products of lipase is favorable to activity rather than depressing. Tanaka [1] has recently studied the use of acids in the cleavage of oil by means of the lipolytic enzym of the castor-oil seed. For every acid there is an optimum amount, and a further addition exerts a retarding influence. The function of the dilute acid is to combine with the basic constituents of the seed. Hence it is uncertain whether or not dilute acids can act as catalyzers for the active form of lipase.

In this and in other researches carried out in this laboratory it has been demonstrated that the acidity of the fat, both crude and extracted, increases after death, whether the chickens are kept at atmospheric temperatures or hard frozen for varying periods of time. During the course of this investigation the presence of active lipase has been demonstrated in the crude fat of chickens hard frozen, at various stages of marketing, and in early and advanced stages of putrefaction. Therefore the post-mortem hydrolysis of chicken fat in situ must be due in large part to the action of lipase.

The following conclusions are reached based on these studies:

(1) Lipase occurs in the crude fat of chickens.

(2) The fat of the fowl immediately after killing shows almost no lipolytic activity. It is therefore probable that the lipase during life exists in its zymogenic form.

(3) As the chicken ages after death, the enzym becomes active and the acidity of the fat increases. These changes occur less rapidly at temperatures below freezing than above the freezing point.

(4) Lipase can resist prolonged freezing for as long a period as 89 months.

(5) From the experiments cited it would appear that the post-mortem hydrolysis of chicken fat depends chiefly upon enzym action.

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[1] J. Coll. Engineering, Tokyo, 5: 25–42; Chem. Abst., 1911, 5: 2004.

II. THE OXIDATION OF CHICKEN FAT BY MEANS OF HYDROGEN PEROXID.

When the forces of nature, including bacteria, molds, yeasts, enzyms, air, light, and heat, act on fats and oils, the various constants undergo changes and an increase in the saponification number is usually accompanied by a decrease in the Hehner number, and vice versa. This phenomenon is due chiefly to the oxidation of the unsaturated acids at the double bonds.[1]

However, when chickens are kept hard frozen both the saponification number and the Hehner number experience a simultaneous change in the same direction.[2] For instance, nine analyses give a mean saponification number of 172.9 and a mean Hehner number of 81.27 for fresh roasters, while three analyses of undrawn roasters, kept hard frozen for 16 months, give a mean saponification number of 194.9 and a mean Hehner number of 91.67, the two constants having increased at the same time.[3] This form of fat decomposition must be due to oxidation of the carbon chain at or near the terminal carbon atoms.

The recent work of Dakin [4] upon the oxidation of the ammonium salts of the saturated fatty acids by means of hydrogen peroxid gave the idea which led to the present research. This investigator discovered that hydrogen peroxid reacts with the ammonium salts of saturated fatty acids at the temperature of boiling water with the evolution of carbon dioxid and the formation of lower fatty acids and of ketones—derived from β-hydroxy acids by loss of carbon dioxid. In some cases aldehydes were also formed. Apparently oxidation occurs at the carbon atom in the β-position to the carboxyl group. It therefore seemed desirable to study the influence of oxidation with hydrogen peroxid upon the chemical constants of chicken fat and of stearic and oleic acids.

The fat was obtained from chickens of known history. All the samples, with two exceptions, were western birds at various stages of their marketing. Sample No. 360 was a chicken kept in a chill room at 32° F. for 24 hours after killing and then analyzed, while sample No. 343 was hard frozen for over a year. The crude abdominal fat was extracted with petroleum ether according to the method

[1] Hepburn, J. Franklin Inst., 1909, **168**: 365, 421; 1910, **169**: 23.

[2] Pennington, U. S. Dept. Agr., Bureau of Chemistry Bul. 115, 68, 73; Premier Congrés International du Froid, Rapports et Communications, 1908, 2: 216; Hepburn, J. Franklin Inst., 1911, **171**: 585.

[3] Results obtained in this laboratory and in course of preparation for publication.

[4] J. Biol. Chem., 1908, **4**: 77, 227.

commonly used in this laboratory[1] and was analyzed. The extracted fat was heated on the water bath for seven hours with a 3 per cent solution of hydrogen peroxid. Six molecules of peroxid were used for each molecule of fat, i. e., two molecules of peroxid for each fatty acid radical. The fat was then separated from the aqueous layer and washed with boiling water until neither fat nor wash water contained peroxid, as was shown by their failure to respond to the potassium-bichromate-sulphuric-acid-ether test (formation of a blue color in the ether). The fat was then filtered through paper in a water oven at 100° C. with the utmost speed, and again analyzed. The methods of analysis were those of the Association of Official Agricultural Chemists.[2] The iodin number was determined by the Hanus method. The analytical data are given in Table III.

TABLE III.—*Changes in the chemical constants of chicken fat produced by oxidation with hydrogen peroxid.*

BEFORE OXIDATION.

Number of sample.	Description.	Iodin number.	Acid value.	Ester value.	Saponification number.	Hehner number.	Percent free acid as oleic.
360	Held in chill room at 32° F. for 24 hours.	64.1	0.3	186.9	187.2	91.66	0.15
350	Western market chicken of known history	66.7	2.2	195.9	198.1	95.20	1.11
2004-1A	Same as No. 350	64.5	1.7	186.3	188.0	90.76	.86
2010-1A	.....do	64.5	1.7	181.6	183.3	88.56	.86
2010-1B	.....do	64.9	1.0	179.7	180.7	88.14	.50
2015-1B	.....do	63.3	1.6	188.1	189.7	91.23	.80
2018-1A	.....do	73.7	1.6	183.2	184.8	89.99	.80
343	Broiling chickens of known history, hard frozen for 12½ months	63.6	3.6	184.8	188.4	90.89	1.81
368	Oleic acid C. P	62.8	.....	.....	193.7	.....	.....
369	Stearic acid C. P	.....	.....	.....	201.3	.....	.....

AFTER OXIDATION.

Number of sample.	Description.	Iodin number.	Acid value.	Ester value.	Saponification number.	Hehner number.	Per cent free acid as oleic.
360	Held in chill room at 32° F. for 24 hours.	64.6	0.5	198.9	199.4	95.95	0.25
350	Western market chicken of known history	61.2	2.4	200.8	203.2	.....	1.21
2004-1A	Same as No. 350	53.1	4.1	205.5	209.6	91.14	2.06
2010-1A	.....do	62.0	2.5	201.8	204.3	94.44	1.26
2010-1B	.....do	66.9	1.8	198.8	200.6	96.16	.91
2015-1B	.....do	54.6	3.6	198.4	202.0	91.14	1.81
2018-1A	.....do	61.6	3.6	202.8	206.4	91.79	1.81
343	Broiling chickens of known history, hard frozen for 12½ months	65.2	3.9	197.2	201.1	94.57	1.96
368	Oleic acid C. P	64.1	.....	.....	192.0	.....	.....
369	Stearic acid C. P	.....	.....	.....	200.2	.....	.....

It will be observed that the acidity always increased; the iodin number usually decreased, although occasionally it increased. The saponification number and the Hehner number almost invariably increased simultaneously, hence dilute hydrogen peroxid at the

[1] Pennington, U. S. Dept. Agr., Bureau of Chemistry Bul. 115, p. 66.
[2] U. S. Dept. Agr., Bureau of Chemistry Bul. 107, Rev., pp. 136-142

temperature of the water bath produced in chicken fat the same chemical change that occurs in that fat in situ during prolonged freezing.

Stearic and oleic acids were also oxidized with hydrogen peroxid. The saponification number of the acids decreased. This change is similar to that undergone by the fat of chickens kept hard frozen for a period of four months, at the end of which time both the saponification number and the Hehner number were lower than in the fat of fresh birds. For example, the fat of 12 chickens hard frozen for four months had a mean saponification number of 146.9 and a mean Hehner number of 66.27.[1]

The fat of fresh chickens does not respond to the fuchsin-sulphurous-acid test of Bianchi;[2] hence it may be assumed that compounds of the aldehyde-ketone family are absent. However, these compounds are present in the fat after oxidation with hydrogen peroxid, because a distinct pink or red color was produced by the oxidized fats when they were treated with fuchsin-sulphurous acid. Oxidized stearic acid also responded positively to the test. Oleic acid before oxidation gave a very faint pink, after oxidation a much deeper pink color. Crude fat from hard frozen chickens contains compounds which restore the color to fuchsin-sulphurous acid, as was shown by tests made on chickens hard frozen for periods of 22, 28, 29, 42, 54, 72, and 89 months. The aldehydes or ketones are usually insoluble in water, and only the fat is colored by the reagent. The biting, aldehydelike odor of long preserved hard frozen chickens has been mentioned by Pennington.[3]

Dakin's work has shown that dilute hydrogen peroxid may oxidize saturated fatty acids with the formation of saturated fatty acids of lower carbon content, ketones, and occasionally aldehydes. In the course of this investigation it has been found that oxidation of chicken fat with hydrogen peroxid gives rise to a simultaneous increase in the Hehner number and the saponification number, while aldehydes are also formed. The increase in the saponification number is doubtless due to the formation of fatty acids which are homologues of those present in the unoxidized fat, but with a slightly lower carbon content. The increase in the Hehner number is explained by the formation of aldehydes and ketones. According to Browne[4] aldehydes play a rôle in the determination of the saponification number and ester value of a fat in which they are present; during the saponification with boiling alcoholic potash, the aldehydes are partly changed into acids which neutralize a portion of the potash. But the rôle is a minor one, as shown by the ester value of oenanthol [n-heptyl aldehyde, CH_3

[1] Pennington. Premier Congrès International du Froid. Rapports et Communications, 1908, 2: 252.
[2] L'Orosi giornal d. chem., 1898, 21: 253.
[3] Yearbook, U. S. Department of Agriculture, 1907, p. 197.
[4] J. Amer. Chem. Soc., 1899, 21: 975.

$(CH_2)_5$ CHO] which has been found to be 15.5. However, the formation of higher aldehydes and ketones, insoluble in water, increases the quantity of insoluble matter in the fat and, therefore, increases the Hehner number.

The changes in the fat of chickens during prolonged freezing are similar to the changes called forth by oxidation of the fat with hydrogen peroxid. The Hehner number and the saponification number increase simultaneously, and aldehydes are formed. The increase in saponification number may, therefore, be ascribed to the formation of slightly lower homologues of the fatty acids of fresh chicken fat, while the increase in Hehner number is doubtless due to the formation of aldehydes and ketones of high carbon content. These changes in the chicken fat in situ are probably produced by the action of enzyms.

During the earlier stages of keeping chickens hard frozen, a simultaneous decrease in both the saponification number and the Hehner number occurs; in explaining this phenomenon, both factors—formation of lower fatty acids and formation of ketones and aldehydes—again must be taken into consideration. The fat probably contains acids which, though insoluble in water, are on the border line between soluble and insoluble; these acids are converted into their lower homologues which are soluble; the Hehner number is thereby decreased, and a tendency is created to increase the saponification number. On the other hand, the higher acids of the fat are oxidized with the formation of aldehydes and ketones, and the saponification number is decreased, as happened in the oxidation of oleic and stearic acids with hydrogen peroxid; this oxidation tends to increase the Hehner number. However, as a resultant of these processes of oxidation, the Hehner number and the saponification number decrease simultaneously. In the course of prolonged freezing the chief rôle is played by the higher fatty acids, which are oxidized and give rise to an increase in both the saponification number and the Hehner number at the same time, as has been described.

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